

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

Kurt W. Kohn, MD, PhD
Scientist Emeritus
Laboratory of Molecular Pharmacology
Developmental Therapeutics Branch
National Cancer Institute
Bethesda, Maryland
kohnk@nih.gov

CHAPTER 3: The Platinum Story: From Imagination to a Major New Anticancer Drug

The previous 2 chapters were about alkylating agents, anticancer drugs that damage DNA by binding tightly (covalently) to DNA bases, especially to guanine. Surprisingly, it turned out that certain molecules centered on a platinum atom can bind and damage DNA in a manner that is rather similar to that of alkylating agents, again especially by attacking at guanines. The anticancer activity of platinum complexes was one of the most surprising and far-reaching discoveries in all of anti-cancer drug research. Particularly remarkable is how that landmark discovery was made.

The first and structurally simplest of the platinum complexes to be discovered was cisplatin, which, with its modified forms, is a mainstay of modern cancer chemotherapy. Cisplatin would not have been discovered in the drug screening programs, because it is an inorganic chemical, while all cancer drug research had been in the realm of organic chemistry, which is based on carbon atoms. Cisplatin is made up entirely of an atom of the heavy metal, platinum, 2 chlorine atoms, 2 nitrogen atoms, and a few hydrogens; there is not a single carbon atom in it (Figure 3.1). Nor would it have been discovered by searching natural products made by animals, plants, fungi, or microorganisms, because platinum is not found in any natural biological system.

Even if heavy metal complexes had been screened for anti-cancer activity, cisplatin could easily have been missed, because the atoms and their configuration have to be just right. For example, cisplatin and transplatin consist of the same atoms and bonds, differing only in whether the 2 chlorines are next to each other (*cis*) or across from each other (*trans*), yet only the *cis* configuration has anti-cancer activity (Figure 3.1).

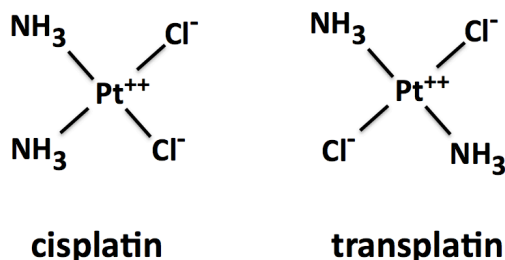


Figure 3.1. Chemical structures of cisplatin and transplatin. The 2 chlorides (Cl^-) and 2 ammonias (NH_3) are arranged in a plane around the platinum (Pt^{++}) atom. They are next to each other (*cis*) in cisplatin and across from each other (*trans*) in transplatin. The platinum atom has 2 positive charges, while the chlorides have one negative charge each; therefore, these platinum complexes are electrically neutral, which allows them to enter cells easily. Both cisplatin and transplatin bind to DNA at guanine-N7 positions. But only cisplatin has the geometry to form DNA crosslinks, and only cisplatin is effective as an anticancer drug.

Discovery by imagination

The clue to the discovery of cisplatin came from an accidental and seemingly bizarre observation by an imaginative and persistent investigator. As noted by Pestko (Pestko, 2002): "cisplatin came from outside the box - so far outside that the box wasn't even visible; it came from a place no one would have dreamt of looking in for an anticancer drug".

As noted by A. Einstein, "Imagination can be even more important than knowledge."

Barnett Rosenberg (1926-2009; his friends called him "Barney") (Figure 3.2) was a biophysicist, working in a small laboratory at Michigan State University with just one assistant. He had graduated from Brooklyn College in 1948 and obtained a PhD in physics from New York University in 1956.

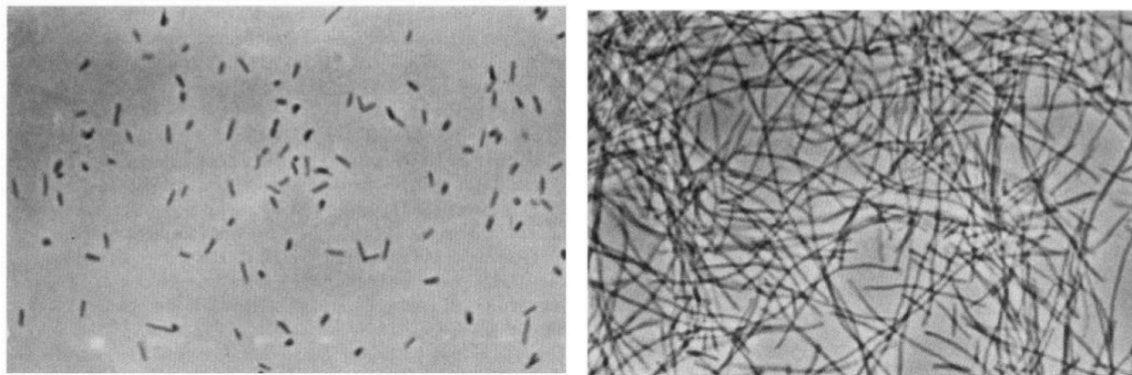
As a biophysicist, a natural way to combine biology and physics in those early days was to examine the effects of electric current on the growth of bacteria; the techniques for such a study were straight forward and easily set up in a modest laboratory. There was little reason to expect any interesting findings.



**Figure 2: Barnett ("Barney") Rosenberg,
discoverer of cisplatin.**

Here is what happened ((Rosenberg et al., 1965), and from what he told me). Barney had a culture of *E. coli* bacteria growing in a growth medium in which he had inserted 2 electrodes connected up so as to pass an electric current through the medium while the bacteria were in there growing and dividing. The electrodes were made of platinum, which was considered to be an inert metal that would not react chemically with anything in the culture. One day, according to Barney, the culture didn't grow. He thought his technician must have forgotten to inoculate the bacteria into the broth. The technician may have known that he did indeed inoculate it, but thought that, well, he'd inoculate it again in the morning. But the broth was perfectly good, he thought, so why bother preparing it again in the morning, so he just put it into the refrigerator. Next day, despite a fresh inoculum of bacteria, still nothing seemed to be growing in that old medium. Now, under similar circumstance, many a researcher would have just dumped the old medium and started fresh. But Rosenberg was curious, so he took a bit of the medium, which was still perfectly clear, as if nothing was growing -- and looked at it under the microscope. What he saw was astonishing.

(Hx Ca Drug Disc Figs cisplatin 160128g.pptx)



Nothing added

Platinum complex added

Figure 3.3. The platinum complex prevented the bacteria from dividing, but allowed them to grow into long filaments. *E. coli* grown without (left) or with (right) an active platinum complex (X600) (Rosenberg et al., 1967a).

E. coli bacteria are normally short rods. But what Rosenberg saw under the microscope were long filaments (Figure 3.3). It seemed that the *E. coli* were growing in length but not dividing. It is impossible to know exactly what was going through his mind when he saw that, but it might have been something like this: There is something in this medium that is preventing the bacteria from dividing, even though it is allowing them to grow in substance, thereby producing those long threads. It has recently been reported, he might have reasoned, that x-rays and agents like nitrogen mustard do exactly that: they let the bacteria grow, but inhibit their ability to divide: they were reported to grow into long threads just like what is here now in this medium. Furthermore, x rays and nitrogen mustard have anticancer activity. So, maybe an anti-cancer substance might somehow have gotten into the medium. But how? The only thing different from the original medium in which the bacteria were growing normally is that there were thin platinum bars (the electrodes) immersed in it and I had passed an electric current through them. But platinum metal is chemically inert. Or is it? What about the electricity that was going through those platinum electrodes? It might have caused some platinum atoms to come off and bind to the ammonia or chloride in the medium.

So that was it! Following up on those ideas, Rosenberg ordered several platinum complexes that were available from a chemical supply company. He soon discovered the active material that prevented the bacteria from dividing while allowing them to grow into long threads: it was indeed a complex of platinum with ammonia and chloride (Rosenberg et al., 1967b); it was in fact the drug that we now call cisplatin (Figure 3.1). In short order, it was tested at the National Cancer Institute and at the Chester Beatty in England for anti-cancer activity in mice. And the results were spectacular! The anticancer activity of the platinum complex was astounding. Moreover, when cisplatin was combined with other drugs, such as

cyclophosphamide, the anti-cancer effects were even more impressive (Rosenberg and VanCamp, 1970) (Woodman et al., 1973).

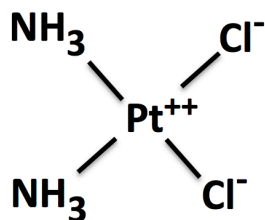
How it works: cisplatin and nitrogen mustard are chemical cousins.

The chemical structures of cisplatin and nitrogen mustard may look very different (Figure 3.4). Nevertheless they both work by crosslinking DNA (Zwelling et al., 1981). The favored site of attack on DNA is the same for cisplatin and nitrogen mustard: the nitrogen at position 7 of guanine.

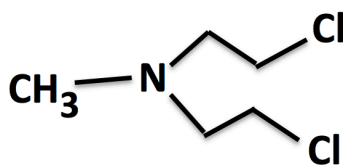
The only similarity between the two structures is that each has 2 chlorine atoms, which indeed is the key to the similarity in their chemical actions. In both drugs, each chlorine can come off, leaving behind a reactive site capable of forming a covalent bond with DNA or proteins. Both drugs have 2 reactive sites whereby DNA can become crosslinked. Although cisplatin, like nitrogen mustard, can produce interstrand crosslinks (Zwelling et al., 1981), cisplatin differs in that it more frequently produces DNA-disabling intra-strand crosslinks (Figure 5).

Crosslink repair is paramount to the drug-treated cell's survival. The cell has highly effective DNA repair mechanisms, which however are not foolproof. Sometimes it leaves the repaired DNA with missing bases, thus producing mutations.

However there was a puzzle about cisplatin: the powerful antitumor action of cisplatin was completely abolished if the 2 chlorine atoms were across from each other (*trans* configuration) rather than next to each other as in cisplatin (*cis* configuration). (Figure 3.4). Leonard Zwelling, who was then a Clinical Associate in my laboratory, decided to investigate this puzzle using the DNA filter elution methods we had been developing at that time to measure both DNA inter-strand and DNA-protein crosslinks (Kohn, 1996). Len's results were quite remarkable: he showed that the *trans* compound produced almost exclusively DNA-protein crosslinks, and lacked the potent cell-killing and mutation-producing actions of cisplatin (Zwelling et al., 1979a; Zwelling et al., 1979b). Evidently, having the active chlorines across from each other (*trans* geometry) was unsuited for DNA crosslinking, whereas it easily crosslinked between DNA and proteins. We were then able to measure the rates of formation and repair of both types of crosslinks. The *trans*-platinum compound then was also useful in our developing a method to quantify DNA-protein crosslinks (Kohn and Ewig, 1979), a method that proved key to the discovery of topoisomerase-targeted anti-cancer drugs (see Chapter 8).



cisplatin



**nitrogen
mustard**

Figure 3.4. The chemical structures of cisplatin and nitrogen mustard look different, but their key chemical reactions are similar. Nitrogen mustard has 2 carbons separating the Cl from the N; that arrangement facilitates the loss of the Cl, leaving behind a reactive group on the molecule (see Chapter 1). Cisplatin too is made reactive by the loss of a chloride, which is facilitated by an abundance of water molecules that replace the Cl^- . In both molecules, two chlorines are essential, because the departure of each one leaves behind an active center, thus allowing 2 reactions to form a crosslink; the 2 chlorines in cisplatin have to be next to each other for the geometry to allow crosslinking of DNA.

(Hx Ca Drug Disc Figs cisplatin 160128g.pptx)

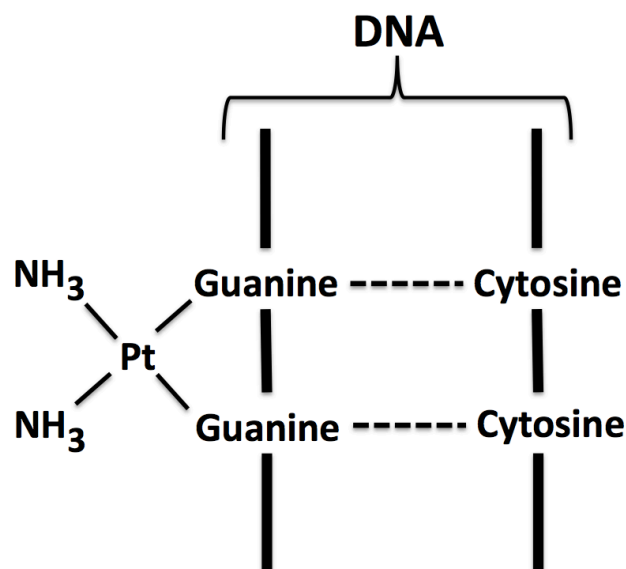


Figure 3.5. After its 2 chlorine ions have come off (and replaced by 2 water molecules), cisplatin can bind to 2 guanines on the same DNA strand. The resulting intra-strand crosslink distorts the DNA, which has to be repaired before the DNA can continue to function; but if the repair fails, the crosslink has lethal potential. Cisplatin's platinum atom (Pt) binds to the same nitrogen atom on guanines (GN7)

as nitrogen mustard does, although nitrogen mustard more often forms *inter*-strand crosslinks (Figure 3.5).

Although it may seem surprising, the 2 DNA crosslinking drugs, cisplatin and cyclophosphamide (a derivative of nitrogen mustard), sometimes are more effective when used together (synergistic) than either of them used alone (Woodman et al., 1973). The reason may be that the crosslinks produced by the two drugs produce different alterations in DNA structure, such as intra- versus inter-strand crosslinks, which are repaired by different molecular systems. Some tumor cells may have a high ability to repair one or the other type of crosslink, while relatively few could repair both types well.

From cisplatin to cell death

There is joy when a patient's cancer responds to chemotherapy. The factors that determine whether the cancer will respond however were complicated and not very well understood. Much attention was paid on identifying factors that gave some degree of predictability of response. Some of them were empirical clinical factors and some were physiology- or molecular-based and all were given much attention as chemical and molecular details were elucidated. A general idea of what is involved is shown in Figure 3.7, which is an overview of some of the main factors that were thought to determine whether a cisplatin-treated cell will live or die.

As the steps governing cancer cell killing were elucidated, that information was used to help improve the clinical effectiveness of the platinum drugs (Galluzzi et al., 2014; Kelland, 2007; O'Grady et al., 2014). The main reaction steps that were found to affect the death or survival of cisplatin-treated cells are diagrammed in Figure 3.7: First, cisplatin has to pass through the cell's plasma membrane to get into the cell, which occurs in part by way of specific channels in the membrane that normally allow essential copper compounds to enter. Particularly important however are other channels, which actively pump cisplatin (normally copper) out of the cell. The intake and export channels affect how much cisplatin is inside the cell (*a* and *b* in Figure 3.7).

(When those particular export pumps are defective, copper is known to accumulate in cells and cause Wilson's disease. One might expect that cisplatin would also accumulate in the cells of Wilson's disease patients, thereby making those cells, whether normal or cancerous more sensitive to the drug.)

Once inside the cell, cisplatin becomes activated by its platinum-bound chlorides being replaced by water molecules (*c* in Figure 3.7). The reason that this replacement activates cisplatin is that the platinum-water bond is weak, and the platinum atom would much rather bind to a nitrogen atom, such as, for example, the one at position 7 of guanine in DNA. That would be the first bond in a prospective

DNA crosslink. The main reason that this chloride-water replacement occurs inside the cell, but not outside, is that the concentration of free chloride is much lower inside the cell than outside in the blood.

When cisplatin has been water-activated (seems odd to put it that way, so scientists instead say “aquated”), what happens next? Sometimes the aquated cisplatin will bind to N7 of DNA guanine, as mentioned above; that toxic reaction however is relatively rare overall, but its impact overrides its rarity. More often, the aquated cisplatin will become inactivated by binding tightly to one of the many sulfur compounds in the cell (such as glutathione, a common sulfhydryl compound, or to metallothione, a metal-binding protein that has a large number of exposed sulfhydryl groups) -- platinum loves sulfur (*d* in Figure 3.7).

The aquated site on the other arm of cisplatin can then proceed to form DNA crosslinks (*e* in Figure 3.7). The resulting DNA damage is detected by a surveillance systems, which signal to the cell’s molecular response systems that danger is afoot (*f* in Figure 3.7). The signals active a remarkable network of logically integrated components that cause the cell to delay DNA replication and cell division in order to give more time for the cell to adapt and to repair the damage before the replication machinery boggles by trying to replicate through a crosslink, or the chromosomes scramble if the cell tries to mitose while its genome is unrepaired. It also puts the cell’s DNA repair machinery on high alert (*g* and *h* in Figure 3.7).

After all that, if DNA crosslinks still remain as the cell tries to move forward in the cell division cycle, a lethal event may occur when the DNA replication machinery encounters a crosslink (*i* in Figure 3.7).

Another process that determines life or death for the cell comes as an output from the damage detection and response network. If the damage persists too long, the system takes no chances and actively suicides the cell -- aficionados call it “apoptosis” (from Greek “falling off,” as in falling off of leaves) (*j* in Figure 3.7). This helps avoid the production of mutated cells that could lead to cancer. However, apoptosis of cancer cells obviously is desirable and is a positive factor for chemotherapeutic response.

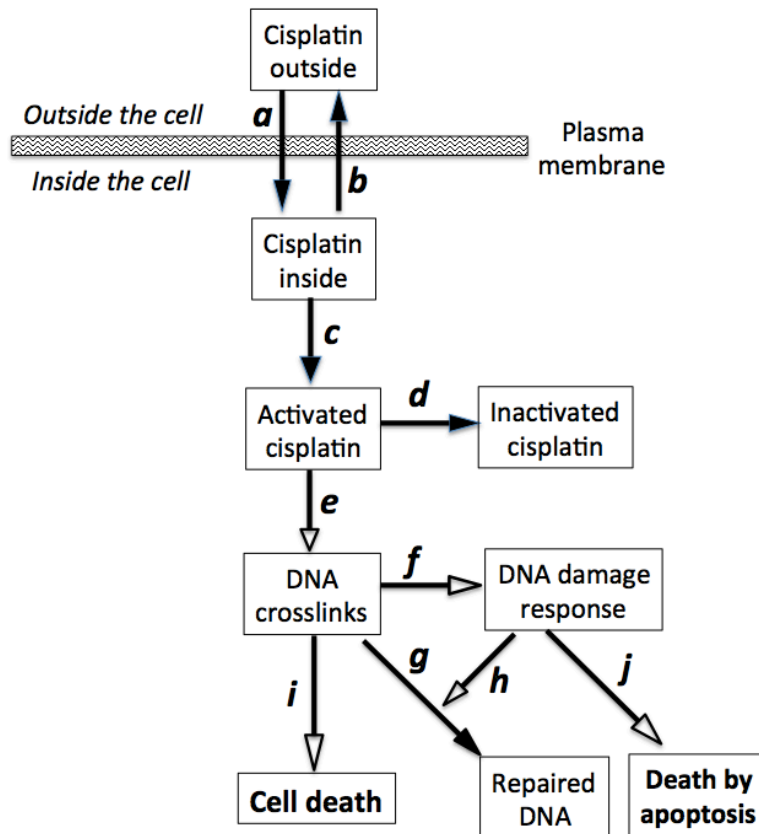


Figure 3.7. Simplified scheme showing factors that were thought to govern the effects of cisplatin on cells. **a** Channels allow cisplatin to enter the cell. **b** Channels that pump cisplatin out of the cell. **c** Cisplatin becomes activated inside the cell by its chlorides being replaced by water molecules (favored inside the cell, where chloride concentration is low). **d** Cisplatin can react with sulfur-containing molecules inside the cell to form inactive products. **e** Cisplatin produces DNA crosslinks. **f** DNA crosslinks stimulate the cell's DNA damage detection and response systems. **g** DNA repair machinery of the cell repairs the crosslinks. **h** DNA damage response system stimulates the cell's DNA repair machinery. **i** Unrepaired DNA crosslinks lead to death of the cell. **j** If the DNA damage repair system fails, it induces the cell to suicide by apoptosis (Galluzzi et al., 2014) (O'Grady et al., 2014).

DaC'16 Fig7 cispt pharkin scheme 150126d

Cisplatin damages the kidney

The main toxicity that limited how much cisplatin could safely be given was damage to the kidneys (Figure 3.8). Cisplatin is actively taken up by cells of the kidney tubules, resulting in deleterious drug concentrations in the cells (Yao et al., 2007). Although the problem was not fully solved, the kidney toxicity was reduced by giving patients lots of fluid and diuretics in order to increase urine flow that would reduce the concentration of the drug in the urine as it flows through the kidney.

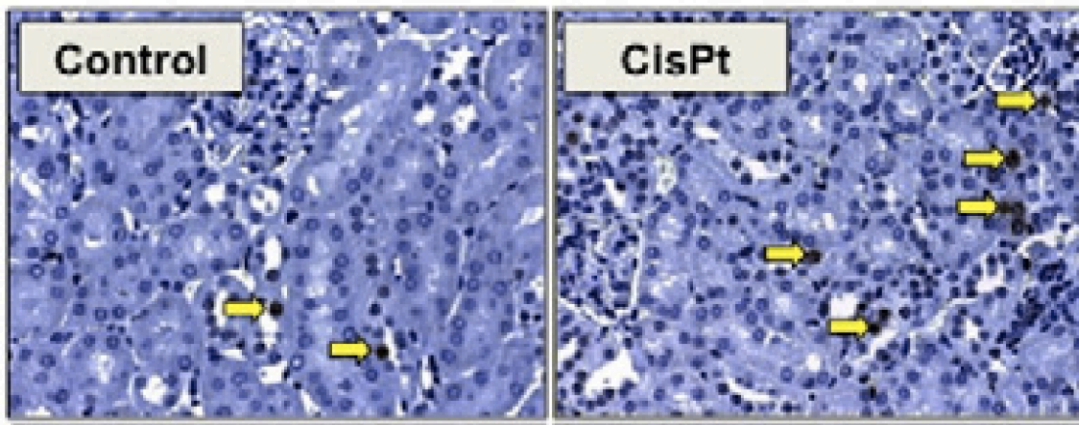


Figure 3.8. Damage to cells of the kidney tubules after a course of cisplatin treatment in mice (Kruger et al., 2016). The yellow arrows point to some of the cell nuclei that have sustained extensive DNA damage (as revealed by staining with antibody to gamma-H2AX: see Chapter 10). After cisplatin treatment (*right*), the number of DNA damaged cells increased 6-fold compared with untreated cells (*left*).

Why some patients refused to take cisplatin.

Cisplatin-containing therapy made most patients so nauseous that many could not stand it. Even entering the hospital where the drug was given was sometimes sufficient to trigger nausea. Many chemotherapeutic drugs can cause nausea, but cisplatin stood out as an extreme case, and the vomiting it induced was sometimes alarming. The cause might be a direct action on certain centers in the brain, but drugs to interfere with that direct action were not yet available. Ordinary available medications controlled nausea that occurred shortly after the drug was administered. However nausea occurred later (perhaps after the drug had entered and affected certain neurons in the vomiting center in the brainstem), at which time it was not easily controlled (De Jonghe et al., 2016; Ishido et al., 2016; Shi et al., 2016).

Treatment of cancer patients with Cisplatin and related drugs.

The testing of cisplatin in tumor-bearing mice, which began in 1968, produced results that were so encouraging that only 3 years later the first clinical trial was begun. That was a remarkably short time between finding anti-tumor activity in mice and approval for clinical trial. Not only was there strong evidence of antitumor activity, but the toxicity and dose-levels were well enough understood to try the drug on human patients. Substantial antitumor effect was first reported in 1974 for testicular and ovarian cancer, and several subsequent clinical trials reported increased survival also in lung cancer patients (Lebwohl and Canetta, 1998). Moreover, cisplatin was unusual because it was not toxic to the blood-forming cells

in the bone marrow. By 1978, the benefit to cancer patients was promising enough to make cisplatin available for general oncology practice.

Modifying the chemical structure of cisplatin in search for better drugs.

Many variations on the cisplatin structure were tested in a search for compounds with increased effectiveness against a greater variety of tumors, with less toxicity, or with lack of cross-resistance to cisplatin (Lebwohl and Canetta, 1998). Two structural relatives became widely used: carboplatin and oxaliplatin (Figure 3.9).

In carboplatin, the two chlorine atoms are replaced by a chemical group that has two carboxyl (COO^-) groups attached to the platinum atom (Figure 3.9). The carboxyl groups activate the platinum in the same way that the chlorides do, that is, by being replaced by water molecules; however, carboplatin is activated more slowly than cisplatin. Another feature is that the two carboxyl groups are part of a structural unit that would favor both carboxyls coming off at the same time; thus the two platinum sites would be aquated together, setting the stage for efficient crosslink production. Carboplatin forms the same kinds of DNA crosslinks as cisplatin, but forms them 10-times more slowly, and 30-times higher drug concentrations are needed. The clinical benefit in terms of increased survival time however was not very different from cisplatin; the main benefit of carboplatin was that it is less toxic; it did not damage the kidneys, and spared the gastrointestinal tract and central nervous system; its dose-limiting toxicity, instead, was suppression of platelet and white blood cell production in the bone marrow (Kelland, 2007; Lebwohl and Canetta, 1998).

In oxaliplatin, like carboplatin, the chlorides are replaced by carboxyl groups in a structural unit. Unlike cisplatin and carboplatin, however, the two amino groups are linked together via a 6-membered ring, a cyclohexyl group (Figure 3.9). Oxaliplatin-mediated DNA crosslinks therefore retain this cyclohexyl structure, which might impair the binding of some DNA repair proteins to the damage site. A notable finding in the clinical experience with oxaliplatin was that, unlike carboplatin, it sometimes was effective in patients whose tumors had stopped responding to cisplatin (Kelland, 2007).

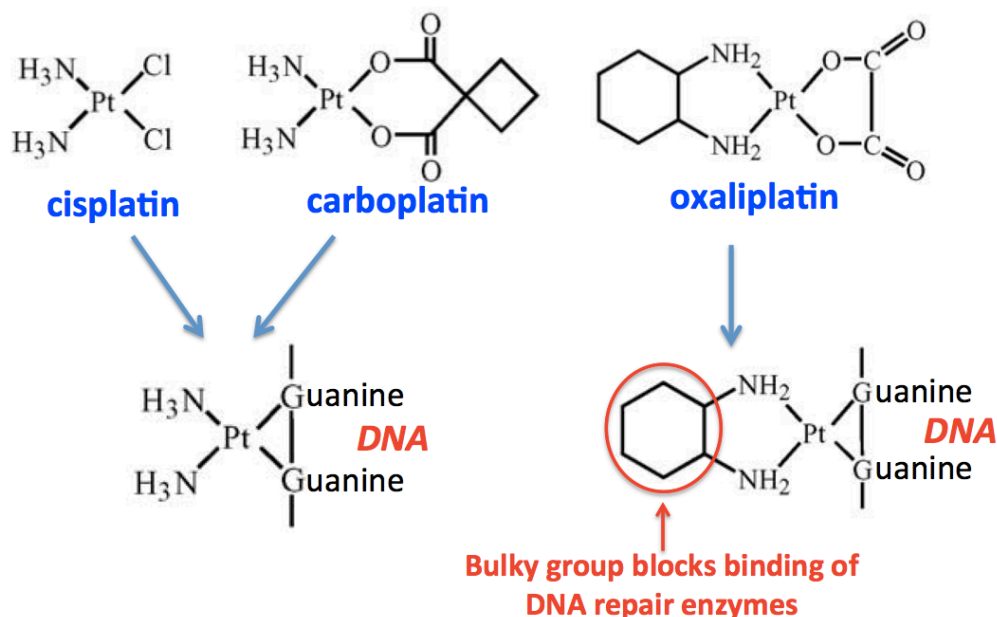


Figure 3.9. The upper row shows the chemical structures of cisplatin and its two relatives that were most commonly used: carboplatin and oxaliplatin. The bottom row shows the structures of the DNA intra-strand crosslinks formed by each of the drugs. The DNA crosslink produced by oxaliplatin differs from the kind produced by cisplatin or carboplatin in that it has an additional 6-membered ring (cyclohexyl group) sticking out from the DNA; this may block the binding of some DNA repair proteins, and may be why oxaliplatin sometimes works against tumors that are resistant to cisplatin or carboplatin (Chaney et al., 2005; Kelland, 2007)..

Cisplatin and the cure of advanced testicular cancer

The greatest benefit of cisplatin was for patients with testicular cancer: cisplatin produced lasting remissions in nearly 80% of the cases, and most of the patients who have the common germ cell type of testicular cancer were cured with a drug combination based on cisplatin (Figure 3.10).

Germ cell cancer of the testis, although relatively rare, was one of the very few types of cancers that could be cured by chemotherapy after having spread (metastasized). What made that possible was the addition of cisplatin to the previous drug combinations that by themselves were much less effective (Einhorn, 1997; Einhorn, 1981; Hinton et al., 2003). The susceptibility of testicular cancer to chemotherapy was in part due to a relatively rapid cell proliferation rate compared to other cancers, and to the fact that testicular cancers rarely become dormant. Tumors with active cell division tended to be susceptible to chemotherapy, as was the case for acute leukemias and choriocarcinoma, and these tumors were often cured (see Chapter ...). Also contributing to their being curable, may be that testicular cancers are nearly unable to repair the DNA damage caused by the drug. (Kelland, 2007).

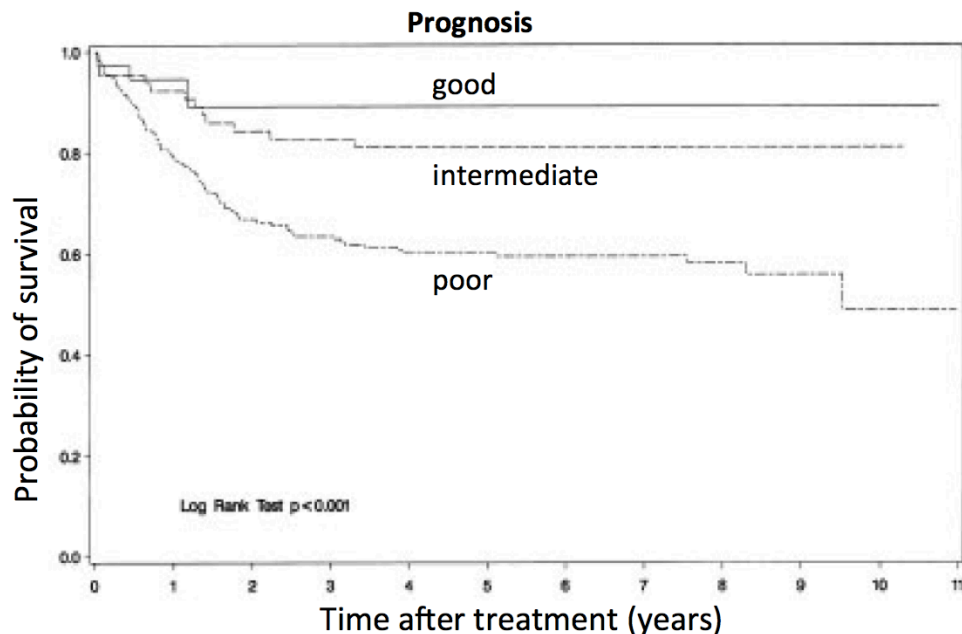


Figure 3.10. A cancer cure. Patients with testicular cancer that had already spread were treated with a cisplatin-based drug combination. The three curves are for patients who were judged at the beginning of treatment to have a good, intermediate, or poor prognosis, based on how advanced their disease was at the time. The results showed that patients whose disease was not highly advanced had greater than 80% chance of remaining alive after 10 years; even highly advanced cases had a 50% chance (Hinton et al., 2003). The curves leveled off with time, showing that patients who survived the first few years were unlikely to die of the disease in the years to come.

(Hx Ca Drug Disc Figs cisplatin 160128g.pptx)

Summary

Cisplatin, which became one of the most useful anticancer drugs, was discovered as the result of an accidental event in a very modest laboratory. Its discovery was due to bold thinking by Barnett (Barney) Rosenberg. The story is remarkable, because there had been no clue that a heavy metal compound, such as cisplatin, could have anticancer activity, and the major drug discovery programs had never considered testing such compounds.

Cisplatin therapy was so promising that great effort was made to overcome the drug's toxicities, and those efforts had significant success. Cisplatin was highly effective and enabled the cure of testicular cancer. The aim then was to modify cisplatin-type therapy so as to find treatments that would be as effective for the common cancers as cisplatin was for testicular cancer. One possibility was that

higher drug doses could be used if toxicity were controlled. Another possibility was to develop better platinum drugs or better drug combinations.

The major toxicity of cisplatin was kidney damage, which however was largely overcome by increased hydration of the patient. Many variations of the cisplatin structure were put in clinical trial, but nothing was found better than the old standbys: cisplatin, carboplatin, and oxaliplatin. Unfortunately, many cancer patients were not helped by any platinum regimens or other available chemotherapy. When tumor regression occurred, it was often brief and survival was extended for but a few months. But complete durable remission did sometimes occur, which gave reason for hope, especially if the exact reasons for the effectiveness against testicular cancer could be worked out.

References

- Chaney, S.G., Campbell, S.L., Bassett, E., and Wu, Y. (2005). Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. *Critical reviews in oncology/hematology* 53, 3-11.
- De Jonghe, B.C., Holland, R.A., Olivos, D.R., Rupprecht, L.E., Kanoski, S.E., and Hayes, M.R. (2016). Hindbrain GLP-1 receptor mediation of cisplatin-induced anorexia and nausea. *Physiology & behavior* 153, 109-114.
- Einhorn, E.H. (1997). Testicular cancer: an oncological success story. *Clinical cancer research : an official journal of the American Association for Cancer Research* 3, 2630-2632.
- Einhorn, L.H. (1981). Testicular cancer as a model for a curable neoplasm: The Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer research* 41, 3275-3280.
- Galluzzi, L., Vitale, I., Michels, J., Brenner, C., Szabadkai, G., Harel-Bellan, A., Castedo, M., and Kroemer, G. (2014). Systems biology of cisplatin resistance: past, present and future. *Cell death & disease* 5, e1257.
- Hinton, S., Catalano, P.J., Einhorn, L.H., Nichols, C.R., David Crawford, E., Vogelzang, N., Trump, D., and Loehrer, P.J., Sr. (2003). Cisplatin, etoposide and either bleomycin or ifosfamide in the treatment of disseminated germ cell tumors: final analysis of an intergroup trial. *Cancer* 97, 1869-1875.
- Ishido, K., Higuchi, K., Azuma, M., Sasaki, T., Tanabe, S., Katada, C., Yano, T., Wada, T., and Koizumi, W. (2016). Aprepitant, granisetron, and dexamethasone versus palonosetron and dexamethasone for prophylaxis of cisplatin-induced nausea and vomiting in patients with upper gastrointestinal cancer: a randomized crossover phase II trial (KDOG 1002). *Anti-cancer drugs*.
- Kelland, L. (2007). The resurgence of platinum-based cancer chemotherapy. *Nature reviews Cancer* 7, 573-584.
- Kohn, K.W. (1996). DNA filter elution: a window on DNA damage in mammalian cells. *BioEssays : news and reviews in molecular, cellular and developmental biology* 18, 505-513.

- Kohn, K.W., and Ewig, R.A. (1979). DNA-protein crosslinking by trans-platinum(II)diamminedichloride in mammalian cells, a new method of analysis. *Biochimica et biophysica acta* 562, 32-40.
- Kruger, K., Ziegler, V., Hartmann, C., Henninger, C., Thomale, J., Schupp, N., and Fritz, G. (2016). Lovastatin prevents cisplatin-induced activation of pro-apoptotic DNA damage response (DDR) of renal tubular epithelial cells. *Toxicology and applied pharmacology* 292, 103-114.
- Lebwohl, D., and Canetta, R. (1998). Clinical development of platinum complexes in cancer therapy: an historical perspective and an update. *Eur J Cancer* 34, 1522-1534.
- O'Grady, S., Finn, S.P., Cuffe, S., Richard, D.J., O'Byrne, K.J., and Barr, M.P. (2014). The role of DNA repair pathways in cisplatin resistant lung cancer. *Cancer treatment reviews* 40, 1161-1170.
- Petsko, G.A. (2002). A christmas carol. *Genome biology* 3, COMMENT1001.
- Rosenberg, B., Renshaw, E., Vancamp, L., Hartwick, J., and Drobnik, J. (1967a). Platinum-induced filamentous growth in *Escherichia coli*. *Journal of bacteriology* 93, 716-721.
- Rosenberg, B., Van Camp, L., Grimley, E.B., and Thomson, A.J. (1967b). The inhibition of growth or cell division in *Escherichia coli* by different ionic species of platinum(IV) complexes. *The Journal of biological chemistry* 242, 1347-1352.
- Rosenberg, B., and VanCamp, L. (1970). The successful regression of large solid sarcoma 180 tumors by platinum compounds. *Cancer research* 30, 1799-1802.
- Rosenberg, B., Vancamp, L., and Krigas, T. (1965). Inhibition of Cell Division in *Escherichia Coli* by Electrolysis Products from a Platinum Electrode. *Nature* 205, 698-699.
- Shi, Q., Li, W., Li, H., Le, Q., Liu, S., Zong, S., Zheng, L., and Hou, F. (2016). Prevention of cisplatin-based chemotherapy-induced delayed nausea and vomiting using triple antiemetic regimens: a mixed treatment comparison. *Oncotarget*.
- Woodman, R.J., Sirica, A.E., Gang, M., Kline, I., and Venditti, J.M. (1973). The enhanced therapeutic effect of cis-platinum (II) diamminodichloride against L1210 leukemia when combined with cyclophosphamide or 1,2-bis(3,5-dioxopiperazine-1-yl)propane or several other antitumor agents. *Chemotherapy* 18, 169-183.
- Yao, X., Panichpisal, K., Kurtzman, N., and Nugent, K. (2007). Cisplatin nephrotoxicity: a review. *The American journal of the medical sciences* 334, 115-124.
- Zwelling, L.A., Anderson, T., and Kohn, K.W. (1979a). DNA-protein and DNA interstrand cross-linking by cis- and trans-platinum(II) diamminedichloride in L1210 mouse leukemia cells and relation to cytotoxicity. *Cancer research* 39, 365-369.
- Zwelling, L.A., Bradley, M.O., Sharkey, N.A., Anderson, T., and Kohn, K.W. (1979b). Mutagenicity, cytotoxicity and DNA crosslinking in V79 Chinese hamster cells treated with cis- and trans-Pt(II) diamminedichloride. *Mutation research* 67, 271-280.
- Zwelling, L.A., Michaels, S., Schwartz, H., Dobson, P.P., and Kohn, K.W. (1981). DNA cross-linking as an indicator of sensitivity and resistance of mouse L1210

leukemia to cis-diamminedichloroplatinum(II) and L-phenylalanine mustard.
Cancer research *41*, 640-649.